METHODS FOR LARGE-SCALE REARING OF THE TOBACCO BUDWORM

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CONTENTS

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METHODS FOR LARGE-SCALE REARING OF THE TOBACCO BUDWORM

By J. R. Raulston and P. D. Lingren, *entomologists, Southern Region, Agricultural Research Service* [1]

Introduction

The ever-increasing demand for large numbers of laboratory-reared insects has necessitated developing more efficient and economical methods of production. The shifting emphasis in insect control utilizing biological entities, such as parasites, predators, and sterile insects, has also created a demand for constantly reliable sources of such insects.

Although *Heliothis* spp. have been reared in the laboratory for many years, few attempts have been made to produce them on a large scale. The most notable work on this has been that of Burton (*2*), Burton and Cox (*3*), and Burton and Harrell (*4*).[2] These researchers described methods whereby procedures normally used for maintaining small laboratory cultures were automated to increase production.

A prime factor in failures to continuously rear *Heliothis* has been the periodic and almost inevitable epidemic outbreaks of viral, microsporidian, or other types of contagious insect diseases, as well as contamination of the larval diet by fungi and bacteria. These outbreaks have normally been followed by annihilation of the cultures, thorough cleanup campaigns, and reestablishment of new cultures. Such procedures result in costly gaps as well as unreliable production.

This publication outlines the methods developed at the Southwestern Cotton Insects Investigations Laboratory, Brownsville, Tex., for rearing tobacco budworms [*Heliothis virescens* (F.)) and describes a pilot-rearing facility at this laboratory that was designed to overcome the problems of disease and microbial contamination.

Artificial Diet

Several larval diets have been developed for maintaining lepidopterious insects (*5*). A popular diet, and perhaps the most successful of the larval diets, is the casein-wheat germ diet described by Vanderzant and others (*11*) and modified by Berger (*1*). With the use of high-quality components, growth and development of insects on this diet are very uniform. This diet, however, is costly and, therefore, undesirable for large-scale production of insects such as the tobacco budworm that consumes relatively large amounts of diet. To reduce its cost, Raulston and Shaver (*7*) added corncob grits as a filler and reduced the agar content from 21 to 4.4 g. per liter. The cost was further reduced by replacing casein with a low-cost soybean flour (*10*). These changes maintained the nutritional quality of the diet and reduced the cost from $0.43 to $0.22 per liter. Dietary components of this diet are listed below. The amounts listed produce about 3.4 liters of diet.

Ingredient	*Amount*
Water (H_2O)	1,500.0 ml.
Potassium hydroxide (4_m)KOH	18.0 ml.
Toasted nutrisoy flour	242.0 g.
Wheat germ	108.0 g.
Wesson's salt	36.0 g.
Sucrose	44.0 g.
L-ascorbic acid	14.5 g.
Methyl-*p*-hydroxybenzoate	5.4 g.
Sorbic acid	3.25 g.
Aureomycin	500.0 mg.

[1] The authors thank the following persons of the Entomology Research Division: M. J. Lukefahr for his encouragement and support of this program; his efforts made possible the construction of the pilot large-scale rearing facility for tobacco budworm; H. T. Dulmage and H. M. Graham for their consultations and criticisms on the design of this rearing facility. J. R. Raulston retired in June 1972.

[2] Italic numbers in parentheses refer to Literature Cited, p. 10.

Ingredient	*Amount*
Vitamin suspension [1]	12.0 ml.
Choline chloride (15 percent)	25.0 ml.
Formaldehyde (10 percent)	15.0 ml.
Acetic acid (25 percent)	40.0 ml.
Agar (in 1,500 ml. H_2O)	15.0 g.
Corncob grits	250.0 g.

[1] Contains the following vitamins per milliliter in water: Calcium pantothenate, 12 mg.; niacin, 6 mg.; riboflavin, 3 mg.; folic acid, 3 mg.; thiamine HCl, 1.5 mg.; pyridoxine HCl, 1.5 mg.; biotin, 0.12 mg.; B_{12}, 0.006 mg.

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FIGURE 1.—Tobacco budworm larval rearing unit consisting of two Fiberglas trays and a polystyrene cell unit.

Larval Rearing

The ability to rear economically large numbers of lepidopteran insects depends upon the efficiency of the larval-rearing techniques. Most laboratory cultures are maintained by using 1- to 2-ounce plastic cups as rearing containers. These containers, however, are unsatisfactory for large-scale rearing because they are costly and present problems in logistics and handling. Roberson and Nobel (*9*) described a larval-rearing technique using Mylar Hexcel [3] as a rearing unit. The technique was refined by Raulston and Lingren (*6*) who reported using a Fiberglas impregnated cardboard Hexcel and a top made of polypropylene cloth that allowed free gaseous exchange. Although this technique presented promise of reducing the cost and logistic problems encountered in the cup-rearing method, occasional outbreaks of microbial contamination made production unreliable. Further refinements, however, described by Raulston and Shaver (*7*) and Shaver and Raulston (*10*) and thorough sterilization of the rearing units have surmounted the contamination problem.

The unit used for larval rearing at the Brownsville laboratory is shown in figure 1. It is composed of two 62- by 31- by 3-cm. Fiberglas trays (Molded Fiber Glass Tray Co., model 607). The upper tray, which is used to cover the unit, is perforated with 0.9-cm. holes to allow adequate ventilation. The cell insert is fabricated from polystyrene light diffusion louvers (Diffusa Lite Co.). It is prepared by gluing four sheets of the louver together with methylene chloride. The resulting unit measures 30.5- by 60.0- by 5.0-cm. and contains 882 1.2-cm. cells.

For larval rearing, 3.4 liters of diet are poured into the Fiberglas tray and the cell is inserted before gelling. The cells are infested with an aluminum implanting sheet that contains drilled depressions corresponding to the cells of the rearing unit (fig. 2*A*). Loose eggs (egg preparation described later) are placed on the planter (fig. 2*B*). The planter, then, is agitated gently to allow the eggs to fill the depressions, and any excess eggs are allowed to roll off. A rearing unit is inverted over it, and the planter is then returned to an upright position, allowing 4 to 6 eggs to fall on the medium in each cell. The unit is covered with a layer of polypropylene cloth (Chicopee Manufacturing Co., fabric No. 6722100) that has been stapled to a sheet of aluminum screen (16 by 18 mesh). This screen prevents the large larvae from perforating the cloth, thus making it reusable. A layer of ½-inch thick polyurethane foam rubber is placed in the perforated top tray, which is then strapped with a pneumatic tension weld strapping tool (Signode Corp., model VFD–306) onto the unit with two strips of Dymax strap (Signode Corp., strap No. 304) (fig. 2*C*). The closed unit offers little chance for microbial contamination from the atmosphere, yet allows free gaseous exchange.

[3] Mention of a propietary product in this publication does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval by the Department to the exclusion of other products that may also be suitable.

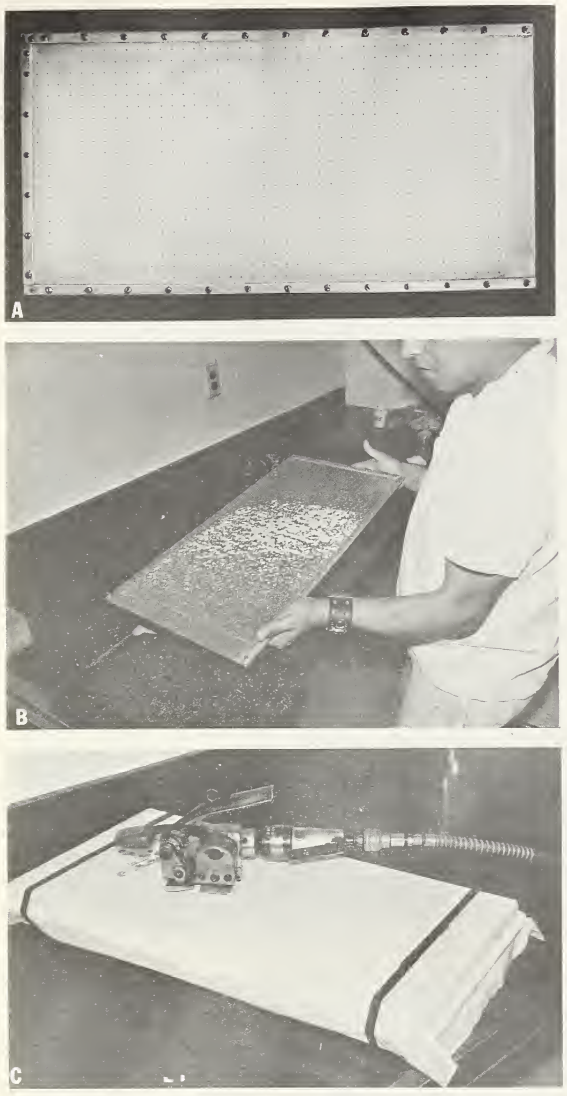

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FIGURE 2.—Egg-implanting technique: *A*, Aluminum egg-dispensing sheet; *B*, placing eggs on dispensing sheet; *C*, closed rearing unit after egg infesting.

The larvae are reared for 19 to 21 days in a room maintained at a temperature of 29.5° C. and a relative humidity of about 50 percent, at which time pupae are harvested. Initially, the opened unit is inverted over an aluminum tray to remove the free pupae (fig. 3*A*), and the remaining pupae are washed from the cell in a tank fitted with two removable grills (fig. 3*B*). These grills are fabricated from ¼-in. steel rods fitted into a 1- by 1-in. angle iron border. The rods of the top grill, spaced at ⅜ inch, allow the pupae to pass through yet retain all large diet particles (fig. 3*C*). Rods of the lower grill, spaced at ⅛ inch, retain the pupae and allow the frass and small diet particles to be washed away (fig. 3*D*).

The pupae, then, are dumped from the grill into a water-filled tray to remove any remaining larvae and exuviae. The unit is washed in water to remove all remaining diet particles. The cell is further cleaned by immersing it in a 20-percent (wt./v.) solution of calcium hypochlorite for 1 hour. This additional cleaning dissolves any adhering silk particles remaining from the puparia. The units are then reassembled and sterilized in an ethylene oxide bedding and mattress sterilizer (American Sterilizer Co.) (fig. 4) for reuse. Ethylene oxide is used to sterilize the unit because the polystyrene cell unit will not withstand steam.

Pupae to be used for propagation of the stock culture are mixed with moist vermiculite and placed in emergence cages (fig. 5*A*). These cages are constructed with a double bottom with 2- by 18-in. strips cut from the upper floor surface to allow air to pass through. No. 8 mesh hardware cloth is mounted over the strips to prevent moths from entering (fig. 5*B*). A small blower fitted with a muslin pillowcase, which serves as a scale filter, is mounted through the double-walled bottom to pull a continuous supply of air through the removed strips (fig. 5*C*). The back of the cage is covered with polypropylene cloth and the moths are attracted to this surface by a light mounted behind the cage. Pupae are held at a temperature of 29.5° C. and 85-percent relative humidity until eclosion.

Moths are collected with a boxlike device that is fitted with a small rheostat-controlled household vacuum sweeper (fig. 6*A*). A 1-gallon ice cream carton, which is used as an oviposition cage, is placed inside the boxlike device and the moths are drawn into it through a 1½-in. vacuum hose. After 20 to 25 pairs of moths have been collected, the cage is moved to the opposite end of the boxlike device where it is fitted with a cheesecloth cover that serves as an oviposition site (fig. 6*B*). These cages are then placed on shelves fitted with scale-filtering blowers

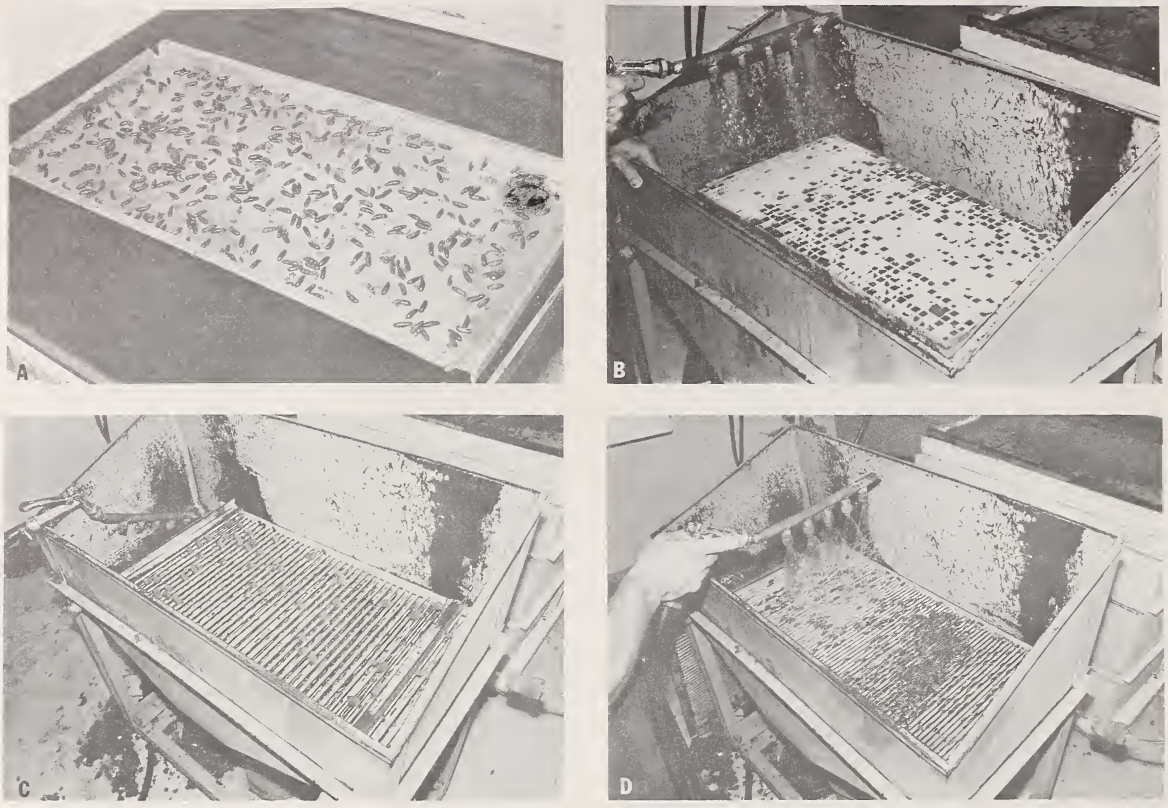

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FIGURE 3.—Pupal harvesting: *A*, Free pupae removed from unit; *B*, pupae being washed from cell; *C*, separation of large diet particle from pupae; *D*, final cleaning of pupae.

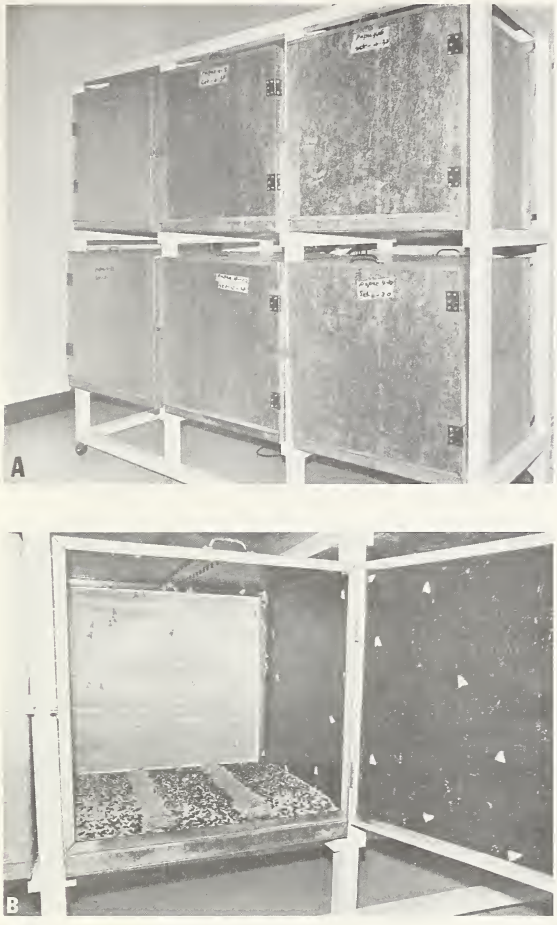
PN-2905

FIGURE 4.—Ethylene oxide gas sterilizer used for sterilizing larval rearing units.

similar to those described by Ridgeway and Whittam (*8*) for use with the pink bollworm (*Pectinophora gossypiella* (Saunders)) (fig. 7).

Adults are maintained at a temperature of 26° C. (day) and 22° (night), a relative humidity of 80 to 85 percent, and a photoperiod of 14 hours light: 10 hours dark. The cheesecloth oviposition pads are changed daily. A 5-percent sucrose feeding solution, which is contained in ¾-oz. plastic cups filled with cotton, is placed inside the oviposition cage. This solution is also changed daily.

These operations are carried out in a hood designed to collect scale freed from the moths while the work is being performed (fig. 8). A large blower mounted below the hood draws air through a filter mounted to the floor. These de-



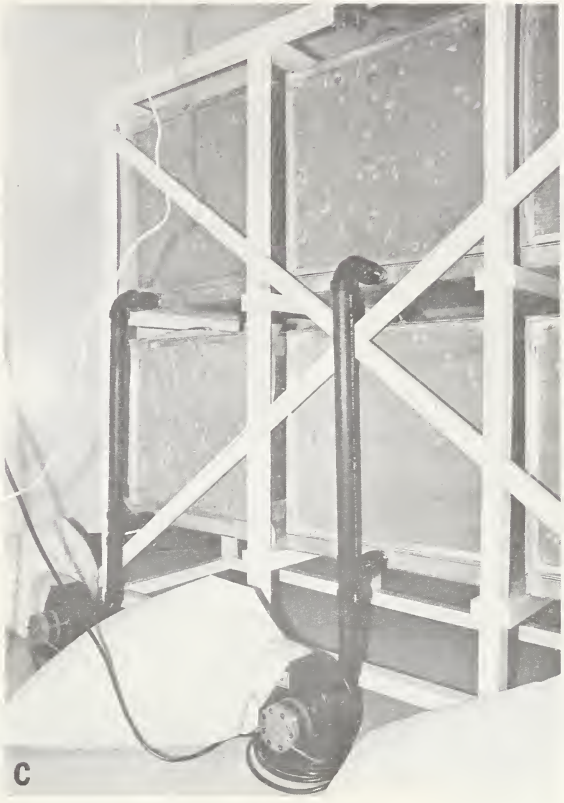


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FIGURE 5.—*A*, Adult emergence chambers; *B*, inner view of emergence chamber; *C*, scale collector mounted behind emergence chamber.

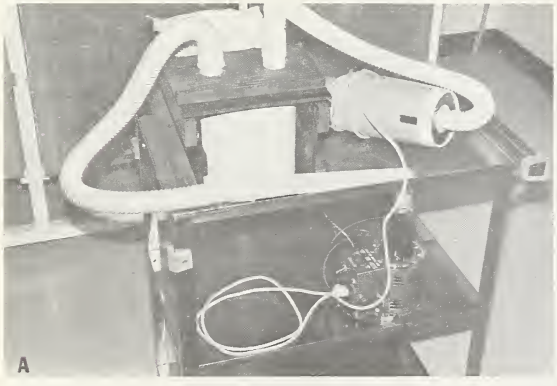

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FIGURE 6.—*A*, Moth-collecting device; *B*, placing oviposition pad on cage containing moths.

vices have effectively reduced, although not eliminated, insect scales in the working areas where adults are handled.

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FIGURE 7.—Oviposition cages setting on scale-collecting pallets.

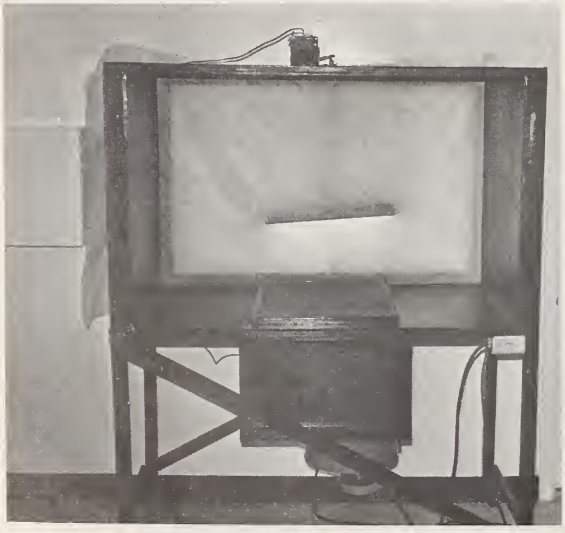

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FIGURE 8.—Scale-collecting work hood for changing oviposition pads on egg-laying cages.

Egg Treatment

After the cheesecloth oviposition pads have been collected, the eggs are surface sterilized. They are then removed from the pads using the following procedure, which is similar to that described by Burton (*2*). The pads are first put into a 0.2 percent solution of sodium hypochlorite for 2 minutes, then they are agitated by hand to remove the eggs. The eggs are collected on a 100-mesh sieve and rinsed thoroughly with tap water. They are then put into a polyethylene squeeze bottle with water and spread over a drying frame covered with polypropylene cloth (fig. 9). After the eggs have thoroughly dried, they are brushed from the frame with a 2-in. paint brush for implanting in the cell units.

General Design of Rearing Facility

The ability to successfully rear large numbers of *Heliothis* spp. depends upon the effective control of microbial contamination and contagious diseases. Development of efficient rearing techniques can be completely nullified by lack of microbial control.

Therefore, a rearing facility had to be designed in which maximum safeguards were incorporated in the basic design of the facility. This design (fig. 10) allows complete isolation of the brood colony from the actual large-scale production facility as well as flow patterns of personnel and materials within the facility that minimize the risk of contamination.

PN–2913

FIGURE 9.—Polypropylene frames for drying treated eggs.

Environmental control in the entire facility utilizes a chilled water system, thus eliminating transfer of air between rooms. Air recycling within each room as well as the incoming fresh air is passed through an absolute filter with a pore size of 0.3 microns. Low ozone-producing germicidal lamps mounted within the fresh-air intake duct are capable of 95-percent kill of all micro-organisms entering with the fresh air.

Figure 11 is a schematic diagram of a typical room within the facility. Recirculated air is drawn from the room through duct (A) by a high-static pressure blower in the cooling coil unit (B). Fresh air enters through duct (C) before the air is conditioned. Two removable prefilters (D) are mounted in the duct immediately in front of the cooling coils to remove any large particles from the air. The conditioned air then passes through the absolute filter (I) before re-entering the room. Exhaust air leaves the room through vent (H) since the room is maintained under positive pressure.

In those areas requiring it, humidity is controlled by the cooling coils and an electric heater mounted above the absolute filter. This system has effectively controlled the presence of airborne contaminants within the facility and has created essentially conventional clean-room conditions for rearing insects. The effectiveness of this system is shown in the following tabulation. The figures in this tabulation are averages of mold and bacterial colonies resulting from 1-hour exposures of sterile nutrient agar in 9-cm. petri dishes in seven rooms of the stock-culture area.[4]

Particulate count comparisons have not been made, however, between our system and those specified for clean-room operations. As a comparison, our old rearing facility, which was maintained under rigid security but had only window-type air conditioners for environmental control, averaged 28.3 mold and 23.8 bacterial colonies per room for 120-day period ending August 1970.

[4] Data were collected for a 69-day period.

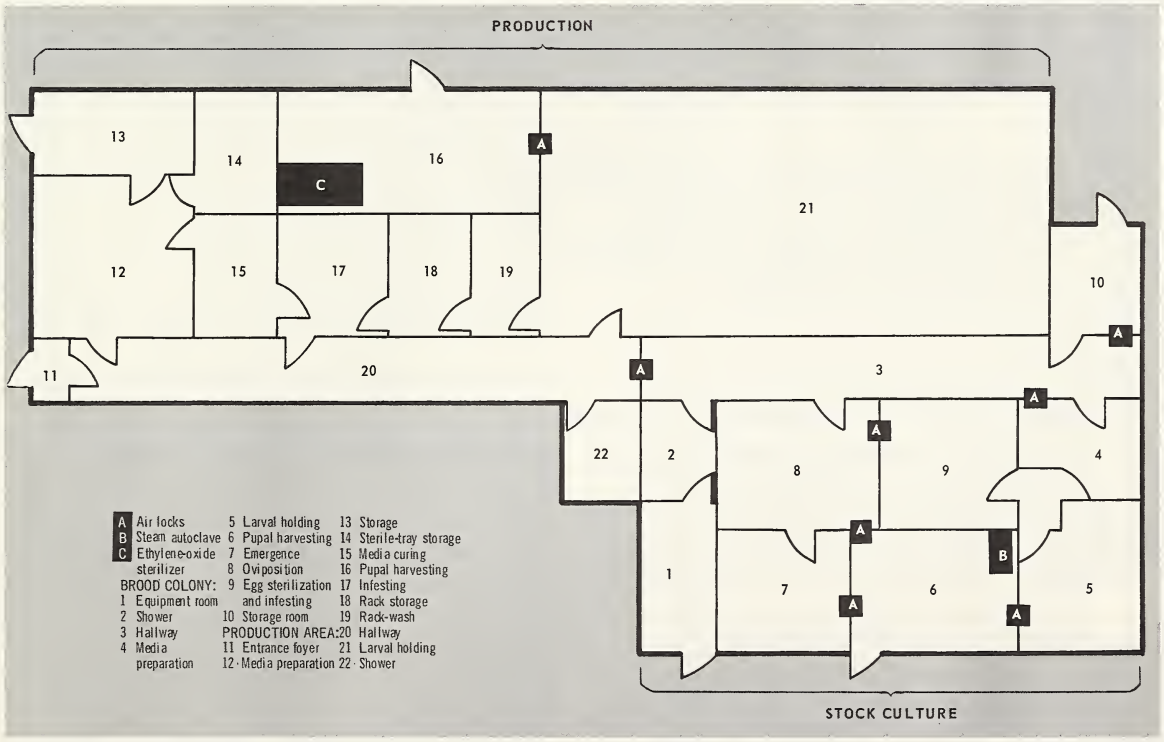


FIGURE 10.—Diagram of Brownsville rearing facility.

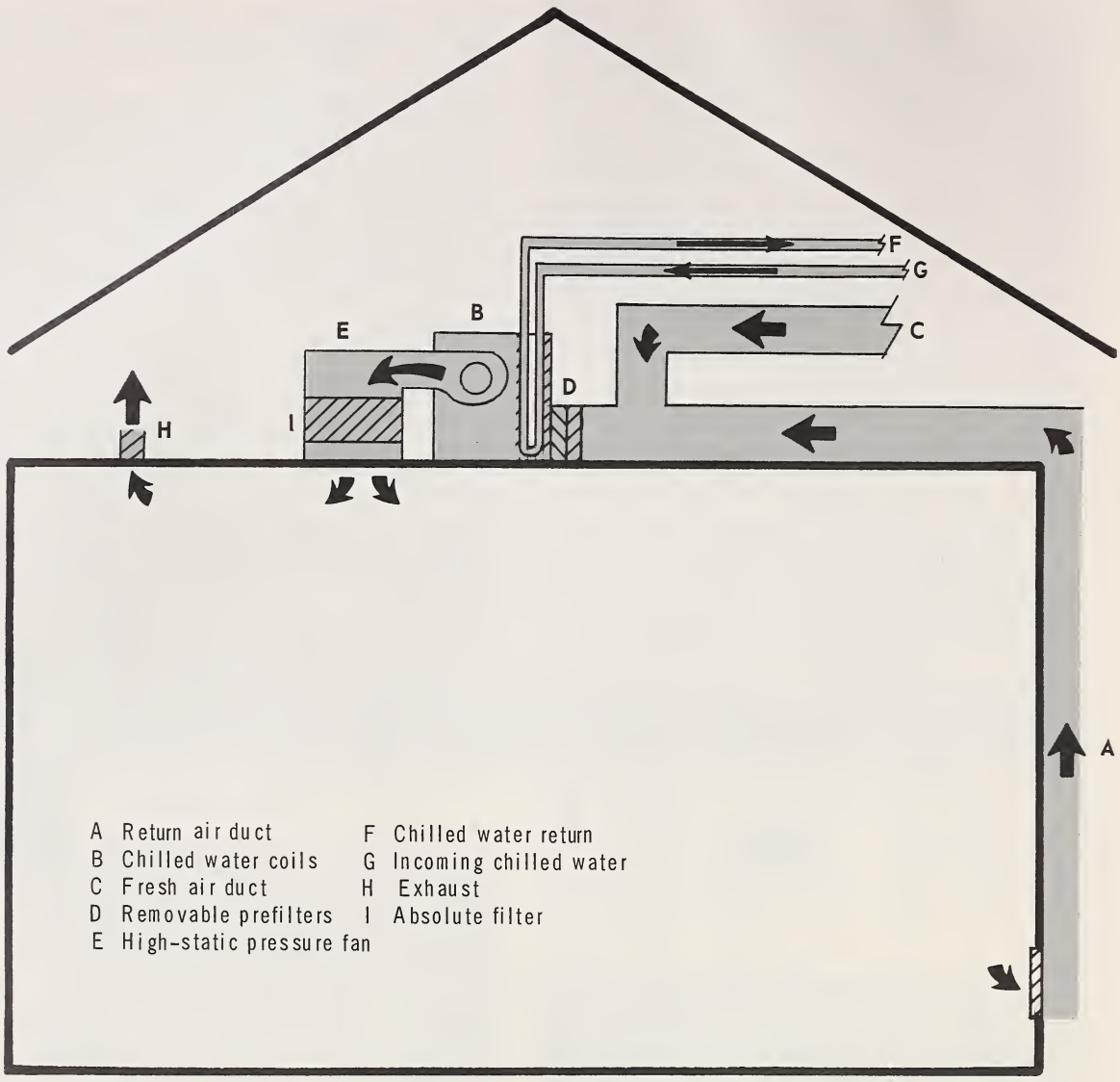
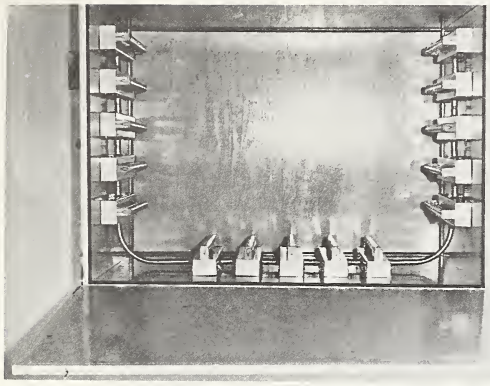


FIGURE 11.—Diagram of a typical room in the rearing facility showing air-conditioning system.

Number of colonies in rooms 1 through 7

Mold	*Bacteria*
0.44	8.0
.01	1.9
0	2.2
.1	5.8
.4	3.2
.1	4.5
.1	3.9

Two technicians maintain the brood colony, designated in figure 10. They enter the building through area 2, where they first shower and change into their laboratory clothes. From this area, these workers enter hallway (3) to their respective work stations. One technician is assigned to rooms 4 (media preparation), 5 (larval rearing), and 9 (egg preparation and infesting), while the other is assigned to rooms



PN–2914

FIGURE 12.—Pass-through air locks containing germicidal ultraviolet lamps.

7 (adult emergence) and 8 (oviposition). Material is transferred between the two areas through air locks fitted with germicidal lamps (fig. 12). A third technician, who enters from outside the building, harvests the pupae in room 6. The pupae are then transferred to room 7 through an air lock. Emergence cages, oviposition pallets, and other reusable material are also cleaned in room 6. These items are then sterilized in a double-door steam autoclave mounted between rooms 6 and 9. All items from the brood-colony area are also transferred into the harvesting and cleanup room through air locks. The cleanup room is maintained under negative pressure. Room 10 serves as a stockroom where necessary supplies are maintained. The stockroom, hallway, and all other rooms, with the exception of those containing insects, are fitted with germicidal lamps. These lamps are illuminated 2 hours each night.

The brood colony produces eggs for use in the production facility. The eggs enter the production facility through an air lock fitted between hallways 3 and 20, which is the only point of contact between the two facilities. Four technicians are assigned to the production facility. One is assigned to areas 12 (media preparation), 14 (sterile-rearing unit storage), and 15 (media curing). The diet is prepared in a stainless steel tank fitted with an overhead Lightnin Mixer, model NM–1 (fig. 13). A single batch of 177 liters, which is sufficient for 50 rearing units, is prepared daily. After the diet has been

PN–2915

FIGURE 13.—Blender for preparing insect diet. Diet is dispensed into the larval-rearing units with a peristaltic pump.

dispensed, the rearing units are stored in area 15 overnight. Another technician is assigned to areas 17 (infesting) and 21 (larval holding). The rearing units are moved from area 15 and the eggs are implanted in area 17, using the techniques described earlier. They are then moved to area 21 and held for 21 days (fig. 14).

After 21 days, the units are passed through an air lock into area 16 where the pupae are harvested and the units cleaned for reuse. Two technicians are assigned to this area. Area 13 is a storage room and areas 18 and 19 are utilized for cleaning and storing the mobile racks used for transporting the rearing units.

Production and Cost Estimates

For a 40-day period ending May 19, 1971, 1,455,857 pupae were produced in the Brownsville-rearing facility, an average of 36,377 per day. Pupal yields have been maintained at a

PN-2916

FIGURE 14.—Larval-holding room for large-scale production of tobacco budworms.

high level for a 6-month period with no major breaks in production.

Based on cost of expendable items, maintenance of building, and wages of personnel involved directly in the rearing process, a cost of $7.09 per 1,000 pupae has been established. This figure does not include wages of supporting personnel involved in administration and supervision.

The large-scale production of tobacco budworms from this facility illustrates the feasibility of rearing *Heliothis* spp. in numbers sufficient for collection of data on such programs as the sterile-male suppression technique and others requiring large numbers of insects. The techniques developed could be easily automated to even further increase the efficiency, thus making it possible to rear this insect in even greater numbers. Only with proper facility design incorporating safeguards against diseases and other types of contamination, however, can such production be maintained.

Literature Cited

(1) BERGER, R.S.
1963. LABORATORY TECHNIQUES FOR REARING HELIOTHIS SPECIES ON ARTIFICAL MEDIUM. U.S. Dept. Agr., Agr. Res. Serv. ARS 33–84, 4 pp.

(2) BURTON, R. L.
1969. MASS REARING THE CORN EARWORM IN THE LABORATORY. U.S. Dept. Agr., Agr. Res. Serv. ARS 33–134, 8 pp.

(3) ——— and COX, H. C.
1966. AN AUTOMATED PACKAGING MACHINE FOR LEPIDOPTEROUS LARVAE. Jour. Econ. Ent. 59: 907–909.

(4) ——— and HARRELL, E. A.
1966. MODIFICATION OF A LEPIDOPTEROUS LARVAE DISPENSER FOR A PACKAGING MACHINE. Jour. Econ. Ent. 59: 1544–1545.

(5) HOUSE, H. L.
1967. ARTIFICIAL DIETS FOR INSECTS: A COMPILATION OF REFERENCES WITH ABSTRACTS. Canad. Dept. Agr. Inform. Bul. No. 5, 163 pp. Belleville, Ontario.

(6) RAULSTON, J. R., and LINGREN, P. D.
1969. A TECHNIQUE FOR REARING LARVAE OF THE BOLLWORM AND TOBACCO BUDWORM IN LARGE NUMBERS. Jour. Econ. Ent. 62: 959–961.

(7) ——— and SHAVER, T. N.
1970. A LOW AGAR CASEIN-WHEAT GERM DIET FOR REARING TOBACCO BUDWORMS. Jour. Econ. Ent. 63: 1743–1744.

(8) RIDGEWAY, W. O. and WHITTAM, D.
1970. A SCALE COLLECTION METHOD FOR PINK BOLLWORM MOTH HANDLING CAGES. Jour. Econ. Ent. 63: 1012–1013.

(9) ROBERSON, J. L., and NOBLE, L. W.
1968. REARING OF TOBACCO BUDWORM IN HONEYCOMB-LIKE CELLS. Jour. Econ. Ent. 61: 331–332.

(10) SHAVER, T. N., and RAULSTON, J. R.
1971. A SOY BEAN-WHEAT GERM DIET FOR REARING THE TOBACCO BUDWORM. Ann. Ent. Soc. Amer. 64: 1077–1079.

(11) VANDERZANT, E. S., RICHARDSON, D. D., and FORT, S. W., JR.
1962. REARING OF THE BOLLWORM ON ARTIFICIAL DIET. Jour. Econ Ent. 55: 140.

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